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Adenylyl Cyclase Activities in Ovarian Tissues. III. Regulation of Responsiveness to LH, FSH, and PGE_1 in the Prepubertal, Cycling, Pregnant, and Pseudopregnant Rat

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ABSTRACT. In the ovaries of prepubertal rats, responsiveness of adenylyl cyclase (AC) to LH, FSH, and PGE_1 is acquired at day 10, coinciding with the appearance of the ability to produce steroids in response to gonadotrophins. The activity and responsiveness of AC on day 11 were similar to those at puberty on day 40 and relatively stable in between.

In the follicles of the cycle, the responsiveness of AC to LH and FSH was poor (*ca* 2-fold stimulation) on metestrus and diestrus, and became high (*ca* 10-fold stimulation) between 1000 h of diestrus and 1000 h of proestrus. Thereafter, the system slowly became desensitized to LH and FSH, being unresponsive by the morning of estrus. Nembutal injected at 1230 h and again at 1500 h on proestrus blocked ovulation and prevented the decline in LH- and FSH-stimulated AC activity.

In the CL of the cycle, the AC was unresponsive to LH on day 1 (estrus), became responsive by the morning of day 2 (metestrus), and maintained responsiveness throughout that day. Thereafter, the responsiveness and basal AC activity declined rapidly.

In the CL of pregnancy, LH-stimulated AC was indistinguishable from that of the CL of the cycle during days 1 and 2, then increased steadily until day 9, showing a transient decrease on days 10 and 11, followed by a sharp rise to maximal activity on days 15 and 16. Thereafter, activity declined as parturition approached.

In the CL of pseudopregnancy (PSP), LH-stimulated AC was very similar to that of the CL of pregnancy during the first 11 days. Thereafter, it decreased coincident with the termination of PSP.

Injections of PRL (100 μg, SC twice daily, from metestrus through estrus, and from proestrus through proestrus) or estradiol-17β (20 μg, SC at 1230 h on metestrus) resulted in "rescue" of the CL-AC system, which remained at metestrus levels when measured on the days of expected proestrus or estrus.

Injections of pregnant mare serum gonadotrophin into prepubertal rats at day 26 (3 IU, iv), induced by day 28 a highly responsive AC system in follicles, with activities equivalent to those found in Graafian follicles on proestrus. By day 29, synchronous ovulation had occurred with a concomitant loss of LH-stimulated AC such as seen in the 1-day-old CL of mature rats.

Our results suggest that the LH-sensitive AC may be indicative of the final development of ovulability of the follicles, and that it may correlate with the functional capacity of CL during various reproductive stages of the rat. (*Endocrinology* **99**: 198, 1976)

IN THE preceding report (1) we showed that desensitization of adenylyl cyclase in rabbit ovarian follicles results from an ovulatory dose of LH or hCG, precedes ovulation, and correlates with the quiescent steroidogenic period prior to and following ovulation. In addition, the pattern of LH-stimulated adenylyl cyclase (AC) activity in corpora lutea (CL) obtained from rabbit ovaries was found to be very similar to that of serum progesterone levels after implantation, suggesting that the AC system may be a rate-limiting step in steroidogenesis (1). It therefore became of interest to explore the AC system in ovarian tissues derived from a different species to determine whether the above observations were limited to the rabbit or whether they might be of a more general nature. The reproductive physiology of the rat has received a great deal of investigation and therefore this animal offers

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a good model for determining the relationship between the functional capacity (steroidogenic) and the gonadotrophin-sensitive AC system in various ovarian tissues.

We present data on the development of the gonadotrophin- and prostaglandin (PG)-stimulated AC systems in the ovaries of prepubertal rats, in antral follicles and the CL of rats exhibiting 4-day estrous cycles, and in the CL of pseudopregnant and pregnant rats, which indicated that most of the findings made in the rabbit can also apply to the rat.

A preliminary account of some of these findings has been reported (2).

Materials and Methods

Animals

Experiments with cycling rats were conducted with 410 female rats obtained at 60 days of age from Charles River (CD, outbred). The rats were housed in an air-conditioned room with lights on from 0500 to 1900 h. Food and water were available *ad libitum*. Vaginal smears were taken 7 days a week by saline lavage at 1000 to 1100 h. Rats were chosen for the experiment after they had shown at least 2 consecutive 4-day cycles. Pregnancy was induced by placing females on the afternoon of proestrus with males known to be fertile. Pregnancy was confirmed by the presence of sperm in the vaginal smear on the next morning. Pseudopregnancy (PSP) was induced by stimulating the cervix with a glass rod at 1700 to 1800 h on the evening of proestrus. The day following mating or PSP induction was counted as day 1.

In the study using prepubertal rats, 98 pregnant rats (Charles River, CD, outbred) were received at 14 to 15 days of pregnancy. The pupping cages were checked at least 2 times each day, and the day on which new litters were found was designated as day 1. Only pups which were delivered prior to 1900 h on day 21 were used. The litters were kept with their mothers until sacrifice, and separated according to sex just prior to sacrifice. Vaginal opening consistently occurred at 40 days of age. Three hundred and ninety-nine pups were used in this study.

For the experiment in which ovulation was induced in immature rats, 130 female rats (Charles River, CD, outbred, received at 20 days of age) were randomly placed into 5 groups when they reached 26 days of age and were injected according to the following regimen: *Group I*: 3 IU PMSG, iv, at 26 days of age (30 rats); *Group II*: 10 IU hCG, iv, at 26 days of age (30 rats); *Group III*: 10 IU hCG, iv, at 26 days of age, and 10 μg estradiol benzoate, SC, at 27 days of age (20 rats); *Group IV*: 0.1 ml of 0.9% NaCl solution (saline), iv, at 26 days of age (30 rats); and *Group V* received 0.1 ml saline, iv, at 26 days of age, and 10 μg estradiol benzoate (EB), SC, at 27 days of age (20 rats). Ten rats from each experimental group were killed on days 27, 28, and 29; the follicles and/or the CL were dissected under a dissecting microscope, homogenized, and assayed for AC activity. Since no follicular growth occurred following saline and EB injections, the follicles could not be dissected from the ovaries, and, therefore, AC activities were measured in homogenates of whole ovaries only (Groups IV and V). For the same reason, the activities from control (untreated) animals were also obtained in homogenates of whole ovaries only.

Pregnant, pseudopregnant, and immature rats were sacrificed by cervical dislocation between 1100 and 1400 h. Cycling rats were sacrificed at 1000 h on each day of the estrous cycle, at 2100 h on the days of diestrus and proestrus, and also at 2345 h on metestrus and proestrus. In each instance, the ovaries were removed and immediately placed into iced Krebs-Ringer bicarbonate buffer (KRB) prepared with one half of the recommended amount of $CaCl_2$ (4). The ovaries obtained from immature rats were dissected free of the oviduct and ovarian bursa and kept cold in KRB until homogenization. The follicles and/or CL were dissected under a dissecting microscope and kept cold in KRB until homogenization.

While the CL formed after cervical stimulation (during pregnancy and PSP) were easily distinguishable from the CL of the previous cycle, some difficulty was encountered in discerning "new" and "old" CL during the cycle. Routinely, 2 classes of CL were dissected during the estrous cycle and assayed for AC activity, one being smaller, more vascular, and with distinct ovulation points, and the other being larger, less vascular, and with less clearly visible ovulation points. The larger, less vascular CL contained an AC which was unresponsive to hormonal stimulation and these CL were considered to be from the previous cycle.

Materials

Estradiol-17β and estradiol benzoate were obtained from the Sigma Chemical Company and were dissolved in peanut oil (1 mg/ml) for injection. Mer-25 (ethamoxytriphetal) was obtained from Merrell-National Laboratories and was dissolved in peanut oil (40 mg/ml) for injection. CB-154 (2-bromo-α-ergocryptine) was a gift from Sandoz Pharmaceuticals and was dissolved in peanut oil (5 mg/ml) for injection.

Adenylyl cyclase assay

Dissected follicles or CL, which had been kept in iced KRB until the dissections were completed, were homogenized in 10 volumes (follicles and CL obtained from ovaries during the cycle and the first 5 days of PSP or of pregnancy) or 20 volumes (CL obtained from ovaries after day 5 of PSP or of pregnancy) of ice-cold medium containing 27% (wt/wt) sucrose, 1.0 mM EDTA, and 10 mM TRIS-HCl, pH 7.5, as described previously (1). Adenylyl cyclase activity in 20 μl aliquots of homogenate was determined as described earlier (1) at 37 C in medium containing 3.0 mM [α-^{32}P]ATP, 5.0 mM $MgCl_2$, 1 mM EDTA, 1 mM cAMP, 20 mM creatine phosphate, 0.2 mg/ml creatine kinase, and 25 mM TRIS-HCl. When present, LH, FSH, and PGE_1 were 10 μg/ml. The final pH of the incubation (10 min) was 7.0. The following number of immature rats supplied sufficient ovarian tissue for one AC assay; 10 for ages 5 to 11, 5 for ages 12 to 18, 3 for ages 19 to 30, and 2 for ages 35 and 40. For experiments with cycling rats, the ovaries from 5 rats supplied sufficient follicular and luteal tissue for one AC assay. The CL obtained from the ovaries of 2 rats were used for each AC assay during pregnancy and PSP.

Unless otherwise stated, all other materials and methods were as described in the preceeding reports of this series (1,3).

Results

Development of adenylyl cyclase in the immature rat ovary

The prepubertal rat ovary first shows distinct biological responses to exogenous gonadotrophin (hCG), such as estrogen secretion and hypertrophy of the interstitial cells (5), at 10 days of age. Prior to day 10, hCG-induced estrogen production is either inconsistent (days 8 and 9) or is absent (day 7). Studies by Lamprecht *et al.* (6), measuring the conversion of [^{3}H]adenine-labeled ATP into [^{3}H]cAMP in a 2 h *in vitro* incubation as a function of age, have demonstrated that LH-induced accumulation of labelled cAMP appeared on day 9 and was maximal by day 15. Although this suggested the appearance of LH-sensitive AC, other effects on either cyclic nucleotide phosphodiesterase or the sizes and distribution of intracellular ATP pools could not be excluded. To determine whether the acquisition of hormonal responsiveness by the ovary could be temporally correlated with a change in AC activity, as assayed directly in a cell-free system, we made daily determinations of basal and LH-, FSH-, and PGE_1-stimulated AC activity from days 5 through 30 and on days 35 and 40. Although AC was present in the ovaries of 5-day-old rats and increased from days 5 to 8, it was unresponsive to LH, FSH, or PGE_1 (Fig. 1). Between days 9 and 10, while basal activity decreased, LH-, FSH-, and PGE_1-stimulated AC activities appeared, reaching levels equivalent to those at puberty (40 days of age) on day 11. These results confirm the observations of Lamprecht *et al.* (6) on the time of appearance of gonadotrophin responsiveness of the cAMP system, and clearly indicate that the development of responsiveness is correlated with the appearance of LH-sensitive AC.

The data presented in Fig. 1 were obtained from pups which were born in our animal quarters. A previous, identical experiment in which female pups were obtained with mothers from a commercial source approximately 4 days prior to use showed large and irreproducible variations in hormonally stimulated AC activity (especially between days 5 and 15).

The abrupt increase on day 10 in the responsiveness of the AC system suggested to us that a specific physiologic stimulus may initiate this event in the prepubertal rat ovary. Previous reports in the literature

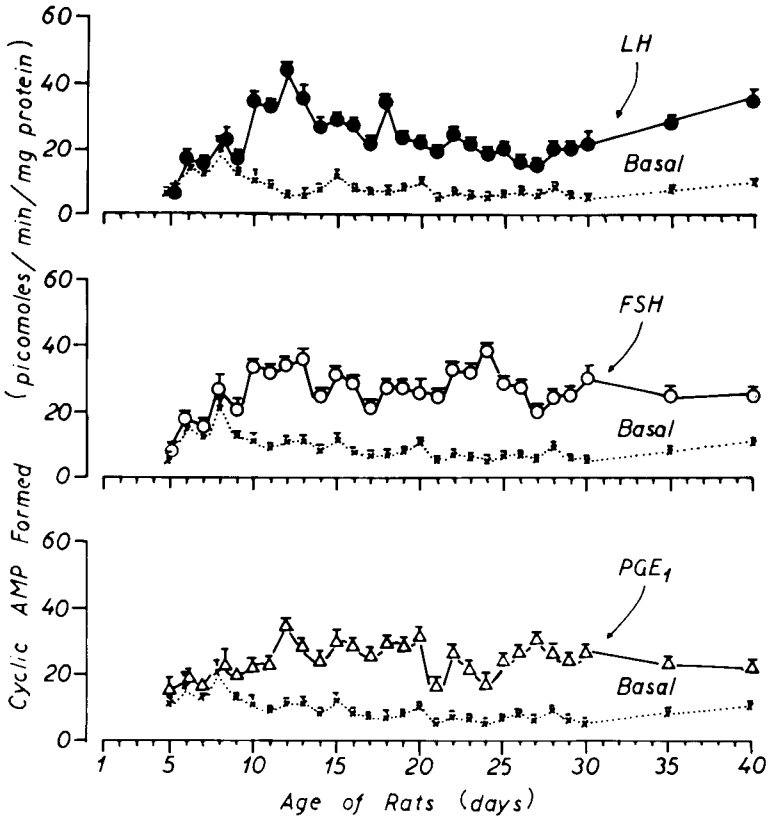

FIG. 1. Adenylyl cyclase activities measured in homogenates of whole rat ovaries obtained from rats 5 to 40 days old. Activity was determined in the absence (basal) and presence of 10 μg/ml of LH, FSH, and PGE_1. Each point represents the mean ± SEM of 3 assays in which ovaries from 10 rats for ages 5 to 11, 5 rats for ages 12 to 18, 3 rats for ages 19 to 30, and 2 rats for ages 35 and 40 were used in each assay. Pregnant mothers, obtained from Charles River (CD, outbred) at 14 to 15 days of pregnancy, delivered the pups used in this study in our animal quarters. Rats were sacrificed between 1100 and 1400 h.

had shown that serum LH, FSH, and estrogen peaked around day 10 (7–9). In an attempt to define the hormonal stimulus responsible for inducing the responsiveness of the AC system, we subjected prepubertal rats to various treatments followed by the determination of hormonal responsiveness of AC: 10 IU hCG were injected SC at 6 days of age, and ovarian homogenates were assayed at 8 days of age; 10 ng of estradiol-17β were injected SC on day 5, and ovarian homogenates were assayed on day 9; 40 μg of MER-25 (an inhibitor of estrogen action) or 50 μg of CB-154 (an inhibitor of PRL secretion) were injected SC on days 6, 7, and 8, and ovarian homogenates were assayed on day 9; and, finally, adrenalectomies were performed on day 7, and ovarian homogenates were assayed on day 9. We were unable either to retard or to advance the responsiveness of the AC system by any of these treatments (not shown).

Variation of adenylyl cyclase in follicles and CL during the estrous cycle

We determined AC activity and the responsiveness of AC to LH, FSH, and PGE_1 in the follicles and CL obtained from adult rats which exhibited 4-day estrous cycles, and found both of these variables to be strongly dependent on the phase of the cycle in which the determinations were made. Follicles obtained on estrus and metestrus contained an AC system which was poorly responsive to gonadotrophin stimulation (Fig. 2). Between 1000 h of diestrus and 1000 h of proestrus, LH- and FSH-stimulated AC activities rose *ca.* 3-fold and basal activity decreased, resulting in a relative stimulation of about 12- and 9-fold for LH and FSH, respectively. PGE_1-stimulated AC activity decreased in parallel with basal activity, resulting in unchanged relative stimulation.

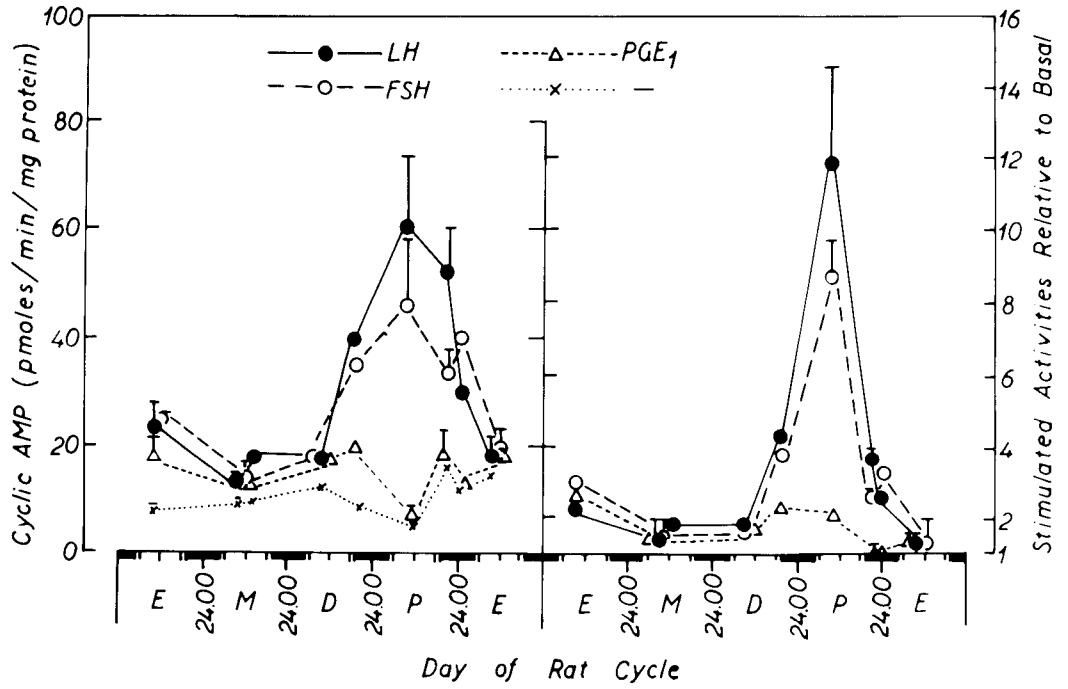

FIG. 2. Adenylyl cyclase activities measured in homogenates of ovarian follicles obtained from rats with a 4-day estrous cycle. When present, LH, FSH, and PGE_1 were 10 μg/ml. Both absolute (left panel) and relative (right panel) activities are shown. Single points represent one assay in which follicles from 5 rats were used. Mean ± SEM is shown where 2 or 3 such assays were performed. For 1000 h on estrus, metestrus, and proestrus and 2100 h on proestrus, n equals 2, 2, 2, and 3, respectively; for all other points, n equals 1.

Following the surge of gonadotrophins in the afternoon of proestrus (10–12), basal AC activity increased, producing a *ca.* 70% decline in the responsiveness of AC to LH and FSH by 2100 h (Fig. 2). Afterwards, the LH- and FSH-stimulated AC activity slowly declined. By 2345 h, LH-stimulated AC had lost about 50% of its activity. On the morning of estrus, the AC in freshly ovulated follicles had become completely desensitized to LH and FSH stimulation.

Adenylyl cyclase activities were low in the newly forming CL on the morning of estrus. Peak LH-stimulated AC activities were reached on metestrus (Fig. 3), falling thereafter to levels equivalent to and below those on estrus.

Patterns of adenylyl cyclase throughout pregnancy and pseudopregnancy (PSP)

Fertile mating on the evening of proestrus did not alter the development of the AC system in the newly formed CL during the first 2 days following mating (Fig. 4). However, the decline in AC activity seen between metestrus and diestrus in the cycling rat was not seen on day 3 of pregnancy; rather the LH-stimulated AC activity continued to increase, gradually reaching a first maximum (2-times metestrus levels) on day 9. Thereafter, that activity declined somewhat, reaching a nadir on day 11, followed by a sharp climb to a second and higher maximum on days 15 to 16. The LH-stimulated AC activity then steadily declined, so that by parturition it was considerably lowered. Throughout pregnancy, FSH- and PGE_1-stimulated AC activities remained low and relatively unchanged.

The induction of PSP on the evening of proestrus produced a pattern of LH-stimulated AC activity that was equivalent to that seen during the first 11 days of pregnancy (Fig. 5). After day 11, in contrast to the ac-

tivities in pregnancy, the LH-stimulated AC activity continued to decline, so that by the end of PSP on day 13, the cyclase was very low and no longer stimulated by gonadotrophins.

Induction of LH-stimulated adenylyl cyclase activity in follicles of prepubertal rats by PMSG and hCG

We determined whether an increase in gonadotrophin-stimulated AC activity paralleled or preceded the induction of ovulation in the immature rat. Follicles dissected from the ovaries of rats which received PMSG on day 26 (Group I, see *Materials and Methods* for procedural details) rapidly acquired a highly responsive LH- and FSH-stimulated AC system. This was clearly evident already on the first day following PMSG (Fig. 6), but was more marked on the second day after PMSG when LH- and FSH-stimulated AC activities had increased to levels equivalent to those measured in the proestrus rat follicle (1000 h, Fig. 2). The induction of this AC system was characterized by an increase in LH-stimulated AC activity, and by a decrease in basal activity. Ovaries on day 29 contained approximately 6 fresh ovulation points per ovary, and no large follicles, indicating synchronous ovulation (presumably due to LH surge on the afternoon of day 28). These ovulated structures contained on AC system characterized by lowered LH-stimulated activity and increased basal activity, similar to that found in the 1-day-old CL of mature rats.

Follicles dissected from the ovaries of rats which received hCG on day 26 (Group II) acquired a highly responsive LH- and FSH-stimulated AC system, characterized by a decrease in basal activity and increases in LH- and FSH-stimulated AC activities, at the same rate as those which received PMSG on day 26; however, the follicles had not ovulated by day 29 as had the follicles in PMSG-injected rats (Fig. 7). Rather, on day 29, the ovaries contained large, preovulatory follicles which possessed an AC system which was highly

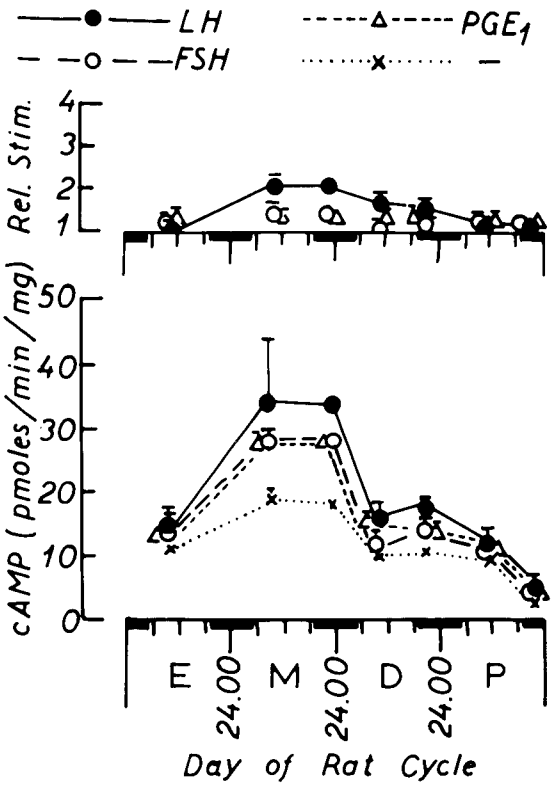

FIG. 3. Adenylyl cyclase activities measured in homogenates in ovarian CL obtained from rats with a 4-day estrous cycle. The rest of conditions are those described in the legend to Fig. 2. For 1000 h on estrus, metestrus, diestrus, and proestrus, n equals 3, 3, 2, and 2; for 2100 h on diestrus and proestrus, n equals 2; and for 2345 h on metesturs, n equals 1.

responsive to LH and FSH, suggesting that no endogenous LH surge had occurred on day 28. The additional injection of EB (Group III) on day 27 induced ovulation by day 29 in hCG-treated rats (Fig. 7). However, in contrast to those of the PMSG-injected rats, the ovaries removed on day 29 from rats which received hCG and EB contained not only fresh ovulation points but also unovulated "preovulatory" follicles and large follicles which had unusually thick walls, suggesting partial luteinization. Both the ovulated (with ovulation point) and unovulated (without ovulation point), partially luteinized structures were pooled and, upon AC assay, showed partial loss of LH-stimulated activity and an increase in basal activity, consistent with the induction of

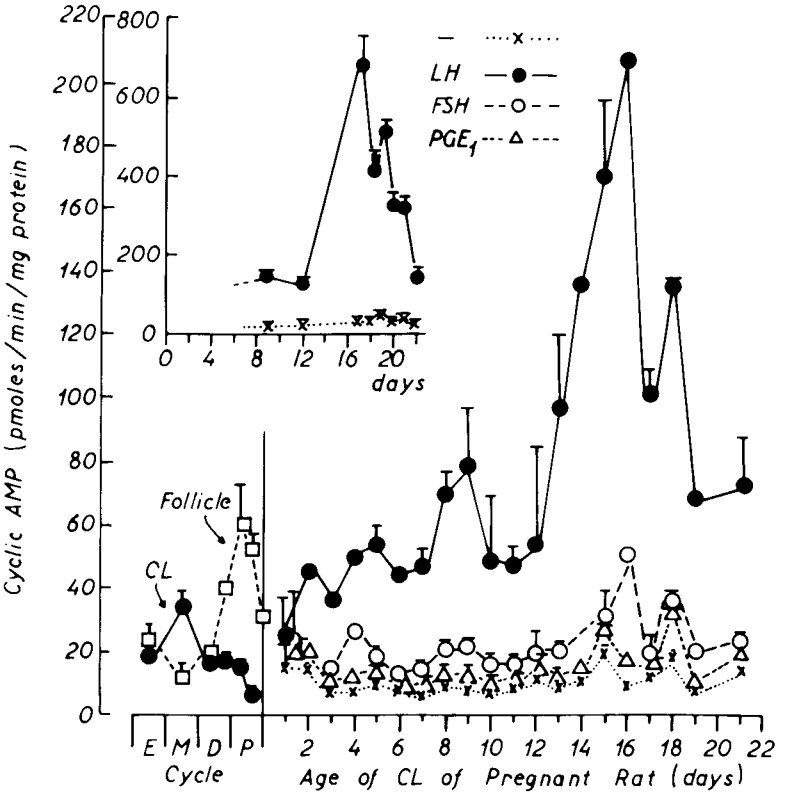

FIG. 4. Adenylyl cyclase activities throughout pregnancy. Basal, LH-, FSH-, and PGE_1-stimulated activities in CL of rats (Charles River, CD, outbred) obtained 1 to 21 days after fertile mating are shown in the right panel. LH-stimulated activity in homogenates of follicles (open squares) and CL (closed circles) dissected from ovaries of rats with 4-day estrous cycles are shown in the lower left panel. When present, LH, FSH, and PGE_1 were 10 μg/ml. Follicle and CL data during the cycle were taken from Figs. 2 and 3. Single points represent one assay using CL from 2 pregnant rats. For days 1, 5, 7, 8, 9, 10, 11, 12, 13, 15, 17, 18, and 21, *n* equals 2, 3, 2, 3, 2, 2, 2, 2, 2, 4, 2, 2, 2, respectively; for all other points, *n* equals 1. *Inset*: Basal and LH-stimulated adenylyl cyclase activities determined in homogenates of CL obtained throughout part of pregnancy from rats of the Charles River CD-inbred strain. Values are mean ± SEM, where *n* was 2, 5, 4, 4, 3, 5, 4, and 4, at 9, 12, 17, 18, 19, 20, 21, and 22 days of pregnancy. CL from ovaries of one rat were used per assay.

desensitization by an endogenous surge of LH.

The LH- and FSH-stimulated AC activity in ovaries dissected from rats which received saline on day 26 (Group IV) or saline and EB (Group V) did not differ from the adenylyl cyclase activity in the ovaries of uninjected rats on days 27, 28, or

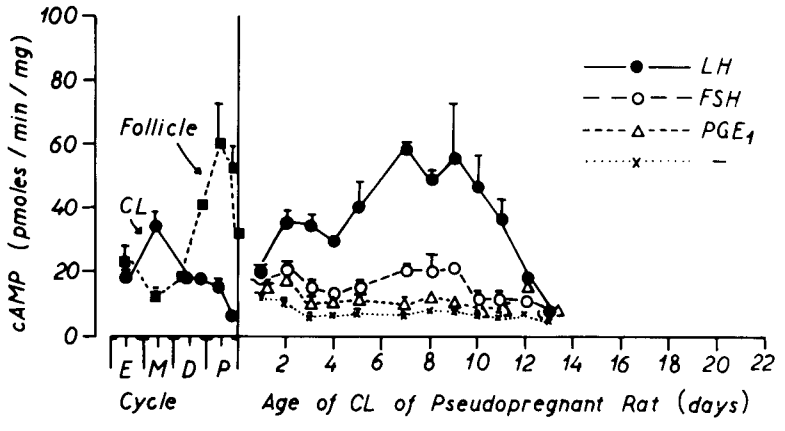

FIG. 5. Adenylyl cyclase activities throughout PSP. Basal, LH-, FSH-, and PGE_1-stimulated activities in CL of rats obtained 1 to 13 days after cervical stimulation are shown in the right panel. LH-stimulated activity determined in homogenates of follicles (closed squares) and CL (closed circles) dissected from ovaries of rats with a 4-day estrous cycle are shown in the left panel. When present, LH, FSH, and PGE_1 were 10 μg/ml. Follicle and CL data during the cycle were taken from Figs. 2 and 3. Single points represent one assay using CL from 2 pseudopregnant rats. Mean ± SEM is shown where 2 to 4 such assays were performed. For days 1, 2, 3, 5, 7, 8, 9, 10, and 11, *n* equals 2, 2, 4, 2, 2, 2, 4, 3, and 2, respectively; for all other points, *n* equals 1.

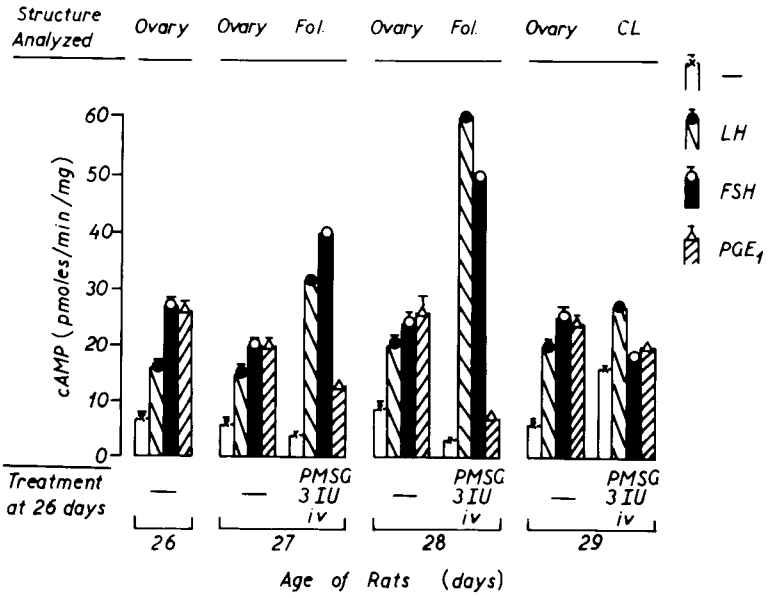

FIG. 6. Adenylyl cyclase activities measured in homogenates of whole ovaries and of ovarian follicles and CL dissected on days 26, 27, 28, and 29 from prepubertal rats treated with PMSG on day 26. The adenylyl cyclase activities of the follicles and CL from the PMSG-treated rats are compared with those of ovaries from rats which received no injections (—). When present, LH, FSH, and PGE_1 were 10 μg/ml. Single points represent one assay in which follicles and/or CL from 10 rats were used. Mean ± SEM is shown where 3 assays were carried out in each of which ovaries from 3 rats were used.

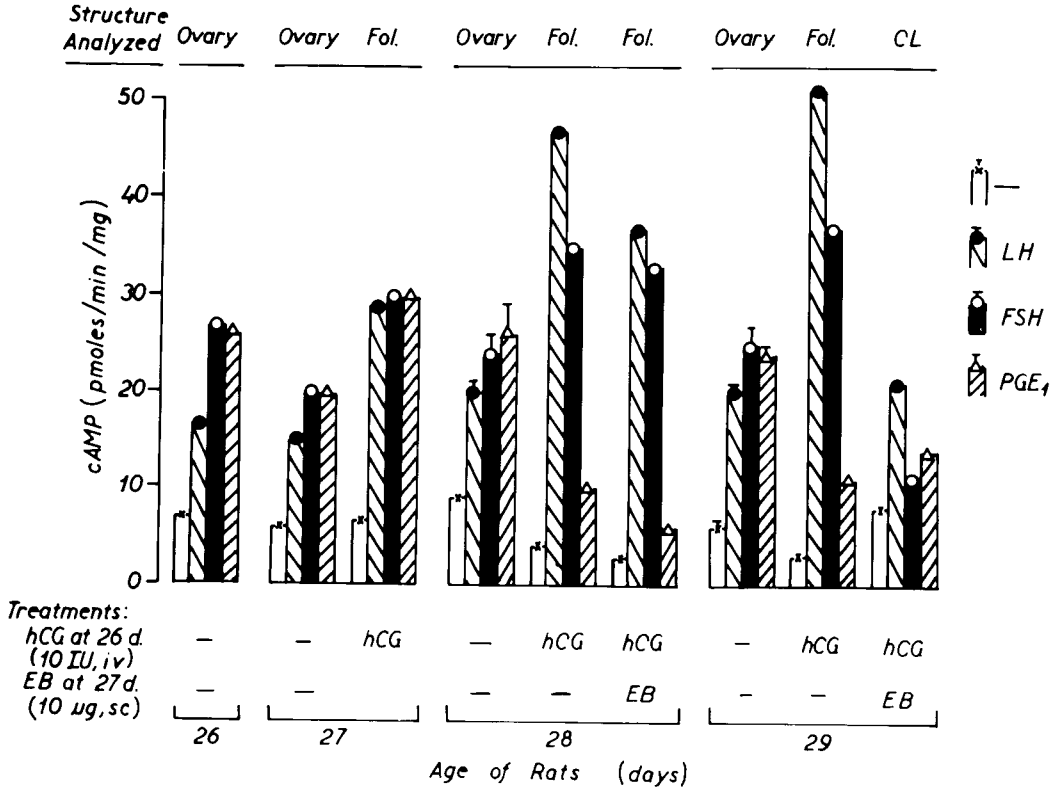

FIG. 7. Adenylyl cyclase activities measured in homogenates of whole ovaries and of ovarian follicles and CL dissected on days 26, 27, 28, and 29 from prepubertal rats treated on day 26 with hCG or on day 26 with hCG and on day 27 with estradiol benzoate. Rest of conditions are those described in legend to Fig. 6.

29 (not shown). In Groups IV and V, none of the rats had ovulated by day 30, and none of the ovaries contained preovulatory-sized follicles.

Effect of estrogen

Everett (13) found that the injection of estrogen into rats displaying 5-day estrous cycles on the second day of diestrus advanced ovulation by 24 h, thereby shortening the cycle length to 4 days. When estrogen is administered to rats exhibiting 4-day estrous cycles, not only is ovulation advanced by 24 h, as in 5-day cycling rats, but also a state of PSP follows in which it appears that 2 sets of functional CL are retained for 7 to 15 days (14,15). We tested the effect of injecting, at 1230 h on metestrus, estradiol-17β (20 μg, SC; Table 1) or EB (20 μg, SC, not shown) and found the AC system in the CL to be more responsive to LH when measured on the days of expected diestrus and proestrus than at any time during the estrous cycle. In fact, the LH-stimulated AC on the day of expected proestrus was equivalent to that of CL obtained on the fourth day of PSP or pregnancy. These CL macroscopically resembled those of PSP and pregnancy in that they were larger and more vascular than CL on the days of diestrus and proestrus. In accordance with the reports of Krey and Everett and Ying and Greep (14,15), the injection of EB advanced ovulation by 24 h. Whether both sets of CL maintained an AC which was responsive to LH and whether the LH-stimulated AC activity in estradiol-induced PSP was equivalent to that of a normal PSP was not investigated.

Effect of PRL

A pseudopregnant state can be induced in the cycling rat with injections of PRL (16,17). We checked to see whether the LH-stimulated AC activity was altered in CL obtained from rats which had received PRL injections. Treatment with PRL (100 μg, in 0.1 ml, SC; 2 times each day) from metestrus through estrus or from proestrus through proestrus produced CL whose AC activity was equivalent to that of CL obtained from rats 3 and 4 days, respectively, after cervical stimulation (Table 1).

Rats that had received PRL injections from metestrus through estrus did not show a typical cornified vaginal smear on the day of expected estrus; rather a leukocytic smear indicative of PSP was obtained, suggesting that ovulation may not have occurred. However, the ovaries from these animals contained freshly ovulated follicles on the morning of estrus, indicating that the LH surge had not been blocked. The freshly ovulated follicles contained unusually low AC activity (about ⅓ that of normal). Whether the freshly ovulated follicles developed into functional CL remains to be determined.

Effect of nembutal

When the endogenous surge of gonadotrophins is blocked on the afternoon of proestrus by an injection of pentobarbital, ovulation is delayed 24 h (18,19). We tested the effect of injecting nembutal (30 mg/kg, ip) at 1230 h and again at 1500 h and found that the LH- and FSH-stimulated AC activities remained elevated (53 and 37.5 pmoles/min/mg protein, respectively) in the unovulated follicles obtained on the next morning (Table 2). Thus, by blocking the proestrus surge of gonadotrophins, we prevented the decline in gonadotrophin-stimulated AC activities coincident with ovulation (Fig. 2).

Variability of results

All experiments presented thus far were carried out with Charles River CD-outbred rats. We attempted to expand the curve of LH-stimulated activity in CL throughout pregnancy, shown in Fig. 4, using (because of temporary unavailability) the CD-inbred strain and obtained the results shown in the inset of Fig. 4. It can be seen that, while the basic pattern of development of the LH-stimulated activity throughout pregnancy was similar, the absolute activities obtained were significantly higher and could

TABLE 1. Effect of estradiol-17β and prolactin (PRL) on luteal adenylyl cyclase activity in rat ovaries at diestrus, proestrus, and/or estrus

	Adenylyl cyclase activity[a] (pmoles/min/mg protein)							
	Metestrus		Diestrus		Proestrus		Estrus	
Treatment	—	LH	—	LH	—	LH	—	LH
None[b]	19.4 ± 2.0	34.2 ± 5.5	9.9 ± 1.8	15.7 ± 1.8	11.7 ± 2.9	14.8 ± 2.8	15.0 ± 2.0	18.5 ± 4.5
Oil[c,d]	—	—	—	—	10.2 ± 1.2	13.7 ± 0.5	—	—
Estradiol-17β[c,e]	—	—	21.9 ± 0.4	63.9 ± 9.9	15.7 ± 0.1	44.6 ± 1.0	—	—
Saline[c,f]	—	—	—	—	13.7 ± 0.9	17.7 ± 1.1	—	—
PRL[c,g]	—	—	—	—	13.9 ± 1.5	35.5 ± 2.8	—	—
PRL[c,h]	—	—	—	—	—	—	11.3 ± 0.5	42.7 ± 2.1

[a] Rats sacrificed at 1000 h.
[b] Mean ± SEM of assays in each of which CL from 5 rats were used. Number of assays on each of the days was 3, 2, 2, and 3 for metestrus, diestrus, proestrus, and estrus, respectively.
[c] Mean ± SD of an assay in which CL from 5 rats were used.
[d] 0.2 ml peanut oil injected SC at 1230 h on metestrus.
[e] 20 μg in 0.2 ml of peanut oil injected SC at 1230 h on metestrus.
[f] Eight consecutive injections of 0.1 ml saline, given 12 h apart, starting at 1800 h of the previous proestrus.
[g] Eight consecutive injections of 100 μg PRL (in 0.1 ml saline), given 12 h apart, starting at 1800 h of the previous proestrus.
[h] Seven consecutive injections of 100 μg PRL (in 0.1 ml saline), given 12 h apart, starting at 0600 h of metestrus.

not be directly related to those obtained with the CD-outbred strain. This points towards the necessity of carrying out complete controls whenever different strains of rats are used in experiments testing for effects of perturbations of the endocrine environment on the AC activities of corpora lutea. We tested the possibility that similarly large variations also occur in different strains of rabbits and found this not to be so. LH-stimulated AC activities in the CL of pregnant California rabbits or "mixed breed" rabbits supplied to us by suppliers in the Chicago area were all found to be in the

TABLE 2. Effect of nembutal on follicular and luteal adenylyl cyclase activity in rat ovaries at proestrus and estrus

	Adenylyl cyclase activity[a] (pmoles/min/mg protein)											
	Proestrus Follicles (2100 h)				Estrus CL (1000 h)				Estrus Follicles (1000 h)			
Treatment on proestrus[a]	—	LH	FSH	PGE_1	—	LH	FSH	PGE_1	—	LH	FSH	PGE_1
None[b]	16.3 ± 4.2	51.9 ± 8.2	38.7 ± 5.5	17.7 ± 5.0	15.0 ± 2.0	18.5 ± 4.5	18.6 ± 3.2	17.3 ± 1.5	—	—	—	—
Nembutal[c]	7.7	45.0	37.6	13.3	—	—	—	—	5.4	53.6	37.5	12.0

[a] Activities were determined in duplicate and agreed within 10%.
[b] Mean ± SEM; n (number of assays) was 3 and 2 for follicles and CL, respectively. Follicles and CL of 5 rats were used for each assay.
[c] The animals were kept under Nembutal anesthesia from 1230 h (initiated with 30 mg/kg, ip) until 1700 h.

range of 110 to 150 pmoles/min/mg for 12-day-old CL, and 100 to 140 pmoles/min per mg for 15-day-old CL, well within the values expected from our studies with New Zealand White rabbits (1).

Discussion

It is now recognized that LH induces many of its effects on Graafian follicles by the stimulation of adenylyl cyclase, and it was therefore of interest to find that follicular structures do not possess responsiveness to LH until shortly before becoming ovulable. Thus, the rat follicle of the cycle acquires its LH-stimulated AC activity just prior to ovulation (Figs. 2, 4, and 5). This phenomenon is not restricted to the rat, since small, unovulable follicles in the rabbit were found to have an AC system that was poorly responsive to LH and FSH (1) and since both hCG-specific binding sites (20) and LH-stimulated AC activities in pig follicles (21) were induced only during the last stages of follicular maturation. It is not known whether the development of LH receptors precedes the development of a potentially gonadotrophin-responsive AC system, or whether the two events occur concurrently. It is conceivable that a potentially gonadotrophin-responsive AC system is already present in small antral follicles and that it is the acquisition of receptors, followed by coupling of the receptor to the adenylyl cyclase, which enables the follicles to respond to the surge of gonadotrophins by ovulating. Related to this question may be the consistent finding that the appearance of LH responsiveness of the AC system coincides with a decrease in basal activity. This is most clearly seen on days 9 to 11 in the prepubertal rat ovary (Fig. 1), but is also very apparent in proestrous rat follicles (Fig. 2), in PMSG- and hCG-primed prepubertal rat follicles (Figs. 6 and 7), and in rabbit follicles (1) as ovulability approached. A similar finding on fluoride-stimulated activity was observed by Robison *et al.* (22) in developing rat brain. One interpretation of these data is that the development of responsiveness of the AC system is associated with a coupling of the receptor to AC and concomitant restriction of basal cyclase activity. A detailed time course determining both the appearance of gonadotrophin-sensitive binding sites and LH-sensitive AC activity may shed some light on the sequence of events, especially if the coupling occurs after the acquisition of LH-binding sites.

The rapid appearance of a highly responsive LH-sensitive AC system between diestrus and proestrus in the cycling rat and in follicles from PMSG- or hCG-primed prepubertal rats (Figs. 2, 6 and 7) may provide a useful model for studying the factors which regulate final maturation of the follicle. Estrogen may be one of the factors which directly effects follicular maturation in rats (23,24). Since it has recently been shown that the injection of highly purified FSH into prepubertal rats results in follicle maturation to ovulability, it will be interesting to determine whether such treatment will produce results similar to those seen in our experiments with PMSG and hCG (25).

LH is thought to be steroidogenic (26–28) in CL of the rat, as in other species, *via* cAMP production. It was therefore of interest to find similarities between our determinations of LH-stimulated AC activity and serum progesterone levels reported in the literature during various reproductive stages of CL in the rat. In the cycling rat, serum progesterone levels are highest at metestrus when the AC system is most responsive to LH stimulation, and are declining by the morning of diestrus when the system is also declining and losing its responsiveness to LH (Fig. 3) (10,12). In the pseudopregnant and pregnant rat, serum progesterone levels and LH-stimulated AC activities exhibit a similar temporal relationship. However, when serum progesterone levels, as determined by Morishige *et al.* (29), are superimposed upon the LH-stimulated AC activities, the correlation is only qualitative. This finding indicates that while there seems to be a relationship between progesterone secretion,

mirrored in serum progesterone levels (30), and cAMP synthesizing capacity, the fine tuning of this relationship still remains to be elucidated. In this respect it is important to keep in mind that progesterone synthesis during pregnancy and PSP is thought to be sustained not simply by LH, which actually decreases in the serum after day 12 of pregnancy (29), but by complexes of luteotrophic hormones: pituitary PRL through day 6, LH and rat placental lactogen from days 7 to 10, and rat placental lactogen after day 11 (31). In fact, in the latter half of gestation, pituitary LH may not necessarily regulate luteal function, since a) hypophysectomy after day 12 does not adversely affect progesterone levels (32), and b) anti-LH after day 12 does not interrupt pregnancy (27). The relationship of the placental lactogenic substances to adenylyl cyclase, if any, remains to be explored.

In view of the, at least qualitative, correlation between LH-stimulated AC activity and the progesterone synthesizing capacity of the CL both in the rat and the rabbit, it was interesting to find that 2 conditions known to extend CL life span also promoted maintenance of LH-stimulated AC activity in CL of the estrous cycle. Thus, injections of either estrogen (on diestrus) or PRL (from proestrus to proestrus or from metestrus to estrus) prevented the expected decline in LH-stimulated AC activity on diestrus so that on proestrus or estrus the adenylyl cyclase activity had remained high (at metestrus levels) and approximated the levels measured on days 4 or 5 of PSP (Table 1).

The following lines of evidence, presented in this report, suggest that the functional capacity of ovarian structures in the rat is associated with and may require adequate levels of LH-stimulated adenylyl cyclase activity: a) the prepubertal rat ovary becomes responsive to gonadotrophins when it acquires a gonadotrophin-responsive AC system; b) follicles from PMSG- or hCG-primed prepubertal rat ovaries rapidly acquire a highly responsive AC system prior to ovulation; c) follicles dissected from cycling rat ovaries on the day of proestrus have acquired an AC system which is highly responsive to LH stimulation; d) CL from pseudopregnant and pregnant rats contain an LH-stimulated AC system which persists throughout the known lifespan of the CL and declines with CL regression; and e) exogenous treatments which prolong the CL life span, such as PRL and estrogen injection or cervical stimulation resulted in the maintenance of CL with an LH responsive AC system.

In conclusion, from the data presented here and in the preceeding report, it would appear that in at least two species, the rat and the rabbit, LH-stimulated adenlyly cyclase activity may be a useful marker for the study of the final stages of follicular maturation (ovulability) and the regulation of the corpus luteum lifespan.

References

1. Hunzicker-Dunn, M., and L. Birnbaumer, *Endocrinology* **99:** 185, 1976.
2. Hunzicker-Dunn, M., and L. Birnbaumer, *Endocrinology* **96:** A110, 1975.
3. Birnbaumer, L., P.-C. Yang, M. Hunzicker-Dunn, J. Bockaert, and J. M. Duran, *Endocrinology* **99:** 163, 1976.
4. Cohen, R. P., *In* Umbreit, W. W., R. H. Burris, and J. F. Stauffer (eds.), Manometric Techniques, ed. 2, Burgess Publishing Company, Minneapolis, 1957, p. 119.
5. Falck, B., *Acta Endocrinol (Kbh)* **12:** 5, 1953.
6. Lamprecht, S. A., U. Zor, A. Tsafriri, and H. R. Lindner, *J Endocrinol* **57:** 217, 1973.
7. Ojeda, S. R., and V. D. Ramirez, *Endocrinology* **90:** 466, 1972.
8. Weisz, J., and P. Gunsales, *Endocrinology* **93:** 1057, 1973.
9. Döhler, K. D., and W. Wuttke, *Endocrinology* **94:** 1003, 1974.
10. Butcher, R. L., W. E. Collins, and N. W. Fugo, *Endocrinology* **94:** 1704, 1974.
11. Nequin, L. G., W. L. Talley, B. G. Mann, and N. B. Schwartz, *Neuroendocrinology* **14:** 65, 1974.
12. Smith, M. S., M. E. Freeman, and J. D. Neill, *Endocrinology* **96:** 219, 1975.
13. Everett, J. E., *Endocrinology* **43:** 389, 1948.
14. Ying, S.-Y. and R. O. Greep, *Proc Soc Exp Biol Med* **139:** 741, 1972.
15. Krey, L. C., and J. W. Everett, *Endocrinology* **93:** 377, 1973.

16. von Berswoldt-Wallrabe, I., H. F. Geller, and U. Herlyn, *Acta Endocrinol (Kbh)* **45:** 349, 1964.
17. Greenwald, G. S., and I. Rothchild, *J Anim Sci* **27** (Supplement 1): 139, 1968.
18. Everett, J. W., *Endocrinology* **44:** 234, 1949.
19. Everett, J. W., and C. H. Sawyer, *Endocrinology* **47:** 198, 1950.
20. Channing, C. P., and S. Kammerman, *Endocrinology* **92:** 531, 1973.
21. Bockaert, J., M. Hunzicker-Dunn, and L. Birnbaumer, *J Biol Chem* (In press).
22. Robison, G. A., M. J. Schmidt, and E. W. Sutherland, *In* Greengard, P., and E. Costa (eds.), Advances in Biochemical Psychopharmacology, vol. III, Raven Press, New York, 1970, p. 11.
23. Ying, S.-Y., and R. O. Greep, *Fertil Steril* **22:** 165, 1971.
24. Ying, S.-Y., and R. O. Greep, *Endocrinology* **89:** 785, 1971.
25. Zeleznik, A. J., A. R. Midgley, Jr., and L. E. Reichert, Jr., *Endocrinology* **95:** 818, 1974.
26. Armstrong, D. T., L. S. Miller, and K. A. Knudsen, *Endocrinology* **85:** 393, 1969.
27. Madhwa-Raj, H. G., and N. R. Moudgal, *Endocrinology* **86:** 874, 1970.
28. Rothchild, I., G. J. Pepe, and W. K. Morishige, *Endocrinology* **95:** 280, 1974.
29. Morishige, W. K., G. J. Pepe, and I. Rothchild, *Endocrinology* **92:** 1527, 1973.
30. Pepe, G., J., and I. Rothchild, *Endocrinology* **93:** 1200, 1973.
31. Morishige, W. K., and I. Rothchild, *Endocrinology* **95:** 260, 1974.
32. Takayama, M., and G. S. Greenwald, *Endocrinology* **92:** 1405, 1973.